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IN THE UNITED STATES PATENT AND TRADEMARK

Inventor

Britta Hardy and Avraham Novogrodsky

Serial No.

08/380,857

Filed

: January 30, 1995

Title

"Immuno-Stimulatory Monoclonal Antibod-

ies"

Group Art Unit

1806

Honorable Commissioner of Patents and Trademarks

SUPPLEMENTAL DECLARATION

RECFINED AUG 2 2 1997

S1R:

- 1. I am one of the inventors in Patent Application No. 08/380,857 (hereinafter: the "application"), and I am the same Declarant who submitted a Declaration to the U.S. Patent Office on February 27, 1997 (hereinafter: "the First Declaration").
- 2. In the First Declaration mention is made to a monoclonal antibody referred to as "BAT mAb". This is the same monoclonal antibody as the BAT-1 monoclonal antibody referred to in the application.
- In the First Declaration, experiments are reported which were made in human-mouse xenograft models, consisting of engrafted human tumors in immunodeficient mice (SCID and nude) (hereinafter: "human-mice xenograft models"). Human-mice xenograft models are acceptable models in the field of drug develop-

AUG

ment for human use, and particularly such cancer models are acceptable in the development of anti-cancer drugs for human use. Furthermore, such models are acceptable as being predictive of the activity of drugs in humans. In view of their predictive value, experiments in human-mice xenografts, are acceptable as being a last step before entering into human clinical trials with a developed drug. Accordingly, the human-mice xenograft models used in my experiments with the BAT-1 monoclonal antibodies, are predictive regarding the activity of BAT-1 monoclonal antibodies in humans.

own knowledge are true, that all made statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment or both, under Section 100 of Title 18 of the United States Code and that such wilful statements may jeopardize the validity of the application or any patent issued thereon.

Dated this 23 day of July

, 199

BRITTA HARDY

18 '97 13:33 cords Reque 3146 SCID C<u>-SKILAUG</u> Records NO. vailable Copy 3146 ₹#1: #2: MICE 470953 SCID MICE 2433 #3: XENOGRAFT 2843 #4: #3 and #4 82 **#5**: HUMAN 692187 #6: #5 and #6 76 **47**: TUMOR 305673 98: #7 and #8 50 3 : :

MEDLINE EXPRESS (R) 1992-1996

1 of 5 Marked Record

P. 4

I: Doxorubicin encapsulated in sterically stabilized liposomes is superior to ree drug or drug-containing conventional liposomes at suppressing growth and

etastases of human lung tumor xenografts. U: Sakakibara-T; Chen-FA; Kida-H; Kunieda-K; Cuenca-RE; Martin-FJ; Bankert-RB D: Department of Molecular Immunology, Roswell Park Cancer Institute, Buffalo,

ew York 14263, USA. O: Cancer-Res. 1996 Aug 15; 56(16): 3743-6

This Journal is Owned by this Library. Holdings According to

Call Number: 1972 v.32-

SSN: 0008-5472

B: Liposomes containing polyethylene glycol-derivatized phospholipids are able to evade the reticuloendothelial system and thereby remain in circulation for rolonged periods. We report here that doxorubicin encapsulated in these terically stabilized liposomes (S-DOX) suppresses the growth of established uman lung tumor xenografts in severe combined immunodeficient (SCID) mice and nhibits the spontaneous metastases of these tumors. The enhanced therapeutic of S-DOX compared to free doxorubicin was demonstrated in two independent human/mouse models. In the first model, S-DOX inhibited the growth of a human non-small cell lung tumor xenograft established orthotopically in the lungs of SCID mice. Treatment of these mice with S-DOX, but not with free irug, suppressed the growth of the tumor in the lung, prevented metastasis from the lung, and enhanced survival percentage. In another model, the human lung tumor is engrafted into gonadal fat pad of SCID mice. Human tumor xenografts prow floridly in this site of engraftment, and the tumor spreads from this provided in the state of the line of the brimary site into the peritoneal cavity and subsequently reaches the liver and lung. In this model, free drug suppressed the growth of the primary tumor but and no effect upon the subsequent spread of the tumor into the peritoneal avity, liver, and lung. In contrast, treatment of the tumor-bearing mice with :-DOX (but not with doxorubicin in conventional liposomes) suppressed the tumor spread to the peritoneal cavity, completely arrested metastasis to the liver and lung, and suppressed the growth of the primary tumor xenograft. This report provides the first evidence that antitumor drugs delivered by sterically stabilized liposomes can arrest the metastasis of human tumor property. N: 96328097

MEDLINE EXPRESS (R) 1992-1996

2 of 5

197 13:34 972 3 9211478P.5 P.83 *** FMRC *RESEARCH CENTER Be vailable Copy

TI: In vitro and in vivo anti-tumour effects of a humised monoclonal antibody against c-erbB-2 product. AU: Tokuda-Y; Ohnishi-Y; Shimamura-K; Iwasawa-M; Yoshimura-M; Ueyama-Y; Tamaoki-N; Tajima-T; Mitomi-T AD: Department of Surgery, Tokai University School of Medicine, Kanagawa,

Japan. SO: Br-J-Cancer. 1996 Jun; 73(11): 1362-5

This Journal is Owned by this Library, Holdings According to

Call Number: 1972 v.26-

ISSN: 0007-0920 LA: ENGLISH

AB: The c-erbB-2 product is thought to be a unique and useful target for antibody therapy of cancers overexpressing the c-erbB-2 gene. In vitro and in vivo anti-tumour effects of a humanised antibody against the extracellular domain of the c-erb8-2 gene product, rhu4D5, were examined. Rhu4D5 was less effective than its murine counterpart, mu4D5, for the direct antiproliferative activity against the c-erbB-2-overexpressing SK-BR-3 cell line. In vivo treatment of severe combined immunodeficient (SCID) mice carrying the c-erbB-2-overexpressing 4-1ST human gastric carcinoma xenografit with 4hu4D5 revealed that the recombinant protein had potent anti-tumour activity. Furthermore, cytotoxicity of human peripheral blood mononuclear cells against 4-1ST was significantly augmented with rhu4D5, but not with mu4D5. These results indicate that rhu4D5 might perform better in patients than predicted from preclinical studies. AN: 96249051

MEDLINE EXPRESS (R) 1992-1996

3 of 5 Marked Record

FI: Establishment of a human B-CLL xenograft model: utility as a preclinical therapeutic model.

AU: Mohammad-RM; Mohamed-AN; Hamdan-MY; Vo-T; Chen-B; Katato-K; Abubakr-YA;

Dugan-MC; al-Katib-A

AD: Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201, USA.

30: Leukemia. 1996 Jan; 10(1): 130-7

ISSN: 0887-6924

N: 96145222

LA: ENGLISH 1B: Chronic lymphocytic leukemia (CLL), a proliferative disease of mature looking B lymphocytes, is the commonest leukemia in western countries. It cemains incurable by available treatment modalities. We report on the establishment of a permanent, EBV-negative, B-CLL line (WSU-CLL) from the peripheral blood of a patient with CLL. The cells grow as suspension in liquid culture, express IgG lambda and other B cell markers and show Ig heavy and light gene rearrangements. Karyotypic analysis shows 15, X, del(3)(p14;p24), t(4;12;12) (q31;q22;p13), t(5;12) (q31;p13), idd(16)(q24)X2, t(18;21) (q12;p12). WSU-CLL forms colonies when grown on soft igar. A xenograft model was established by injecting the WSU-CLL cells subcutaneously (s.c.) in severe combined immune deficient (SCID) mice. When the continuously transplanted in vivo to other SCID mice, the success rate was look with a doubling time of 7.3 days. The CLL-SCID xenograft model was used to test the efficacy of selected standard chemotherapy drugs and new therapeutic igents against WSU-CLL. The cell line and the xenograft described can be used is a model to facilitate the development of new therapeutic agents against CLL .n man.

. I: The urokinase inhi

rostate tumor in SCID mice. U: Billstrom-A; Hartley-Asp-B; Lecander-I; Batra-S; Astedt-B D: Pharmacia Oncology Immunology, University Hospital, Lund, Sweden.
D: Int-J-Cancer. 1995 May 16; 61(4): 542-7

This Journal is Owned by this Library. Holdings According to Call Number: 1972 v.9-

SSN: 0020-7136

B: Malignant cells possess a high degree of proteolytic activity in which the lasminogen activator system plays an important role. An increased expression f urokinase type plasminogen activator (uPA) is of significance for egradation of the extracellular tumor matrix, facilitating invasiveness and rowth. Inhibition of the active site of uPA makes it possible to evaluate the ignificance of uPA in tumor growth. We report here experiments on a PA-producing human prostate xenograft (DU 145) using a competitive inhibitor f uPA, p-aminobenzamidine. In vitro experiments with DU 145 cells showed that -aminobenzamidine caused a dose-dependent inhibition of uPA activity. DU 145 ells were inoculated s.c. in SCID mice and, once tumors were established, reatment with p-aminobenzamidine added to drinking water was started and asted for 23 days. Mice receiving 250 mg/kg/day of p-aminobenzamidine showed a :lear decrease in tumor-growth rate compared to the non-treated mice, resulting in 64% lower final tumor weight. In addition, upA-antigen levels in the membrane fractions of DU 145 tumors from p-aminobenzamidine-treated mice were ound to be decreased by 59%. We also show that p-aminobenzamidine has an inti-proliferative effect in cell culture at low cell number, correlating with dose-dependent decrease in uPA production. In conclusion, we show that a .ow-molecular-weight uPA-inhibitor, p-aminobenzamidine, has a growth-inhibitory effect on a solid uPA-producing tumor.

MEDLINE EXPRESS (R) 1992-1996

5 of 5 Marked Record

[]: Dexamethasone reduces the interstitial fluid pressure in a human colon

idenocarcinoma xenograft.

AU: Kristjansen-PE; Boucher-Y; Jain-RK

D: Edwin L. Steele Laboratory, Harvard Medical School, Massachusetts General

jospital, Boston 02114.

30: Cancer-Res. 1993 Oct 15; 53(20): 4764-6

This Journal is Owned by this Library, Holdings According to

Call Number: 1972 v.32-

ISSN: 0008-5472

N: 95279003

AB: The effect of dexamethasone on interstitial hypertension was evaluated in a human colonic adenocarcinoma. Two weeks after transplantation of the tumor line LS174T into SCID mice, recipients with tumors > 8.5 mm in diameter received one daily injection i.p. on days 1-4, at five different doses in the range of 0.3-30 mg/kg. Controls received saline. The interstitial fluid pressure (IFP) was determined in all tumors pretherapeutically on days 1, 4, and 7. A total of 68 tumors were examined, and in an additional group of 22 mice, the effect of 4-day dexamethasone therapy on blood pressure was evaluated. In the 3-, 10-, and 30-mg/kg dose groups a significant reduction in IFP was found, comparing treated versus controls and individual measurements from day 1 versus day 4. No rereas 0.3 mg/kg digger valiable consone therapy, and retreatment related ightly increased by dexamethasone therapy, and retreatment the reversible langes in tumor sizes were observed. Our findings indicate that the reversible crease in tumor IFP by dexamethasone is an effect of a reduced microvascular immeability and vascular hydraulic conductivity in the tumors.

1: 94006268

CURRICULUM VITAE

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A. EDUCATION

1959-1963 Secondary School "Kol Israel Haverim"

(Alliance), Ramat Aviv, Tel Aviv

1963-1967 B.Sc. in Microbiology, Bar Ilan University, Ramat Gan,

Studied Microbiology and Biochemistry

1967-1969 *M.Sc. in Microbiology* (with distinction)

Bar Ilan University: Thesis entitled:

The effect of FSH on RNA synthesis in immature mice ovaries

Supervisor: Prof. B. Lunenfeld.

1970-1975 *Ph.D.* student at the Feinberg Graduate School

and the Dept. of Biological Ultrastucture at the Weizmann Institute of Science, Rehovot, Israel

Supervisor: Prof. David Danon.

Oct. 1975 Received Ph.D. degree at the Weizmann Institute

of Science, Rehovot. Thesis entitled:

Structural and functional differences in macrophages of mice

deficent in antibody production.

B. FURTHER STUDIES

Sept. 1976-July 1979 Post Doctoral Fellow in Hematology, Stanford University,

School of Medicine, Dept. of Medicine, CA., USA

Research on "The role of spectrin and actin in the red blood

cell membrane."

Supervisor: Prof. Stanley Schrier, Head of Hematology.

Awarded: Chaim Weizmann Fellowship for Post Doctoral Studies in the

U.S.A.

Awarded: Fellow of Hematology. Stanford University Medical School.

Oct. 1984-July 1985 Visiting Research Fellow at Stanford University Medical School

Dept of Pediatrics.

Research on monoclonal antibodies to endothelial cells.

Dean's Fellowship

Awarded: Fellow of Medicine

C. EMPLOYMENT

1968-1969 Research assistant at the Bar Ilan University, Ramat-Gan, Israel

Dept. of Microbiology

Sept. 1975-Sept 1976 Research Scientist, Department of Biological Ultrastructure

Weizmann Institute of Science, Rehovot, Israel

Sept. 1976-July 1979 Post doctoral Fellow at Stanford University School of Medicine

Department of Medicine, Hematology Division,

Stanford, CA., USA Head, Prof. S.L. Schrier

Sept. 1979-1980 Dept. of Chemical Immmunology

The Weizmann Institute of Science, Rehovot, Israel

Laboratory of Prof. Edna Mozes

Jan 1980-Sept. 1985 Rogoff Medical Research Institute, Beilinson Medical Center

Petah Tivka, Israel.

Head of Lipidosis Unit for Reseatrch and diagnosis of Gaucher

and Niemann Pick diseases. Received Tenure in Kupat Holim

Oct. 1985- July1986 Stanford University, Medical Center, Dept. of Peditatrics, CA., USA

Dean's Fellowship

Sept. 1985-July 1988 Rogoff Medical Research Institute, Beilinson Medical Center,
Petah Tikva, Israel. Head of Unit for development use of monoclonal
antibodies in research and treatment of disease.

Sept. 1988-July 1989 IDEC Pharmaceutical Corp., Mountian View, CA, USA.

Research on shared idiotypes in B lymphoma and production of anti-idiotypes for the treatment of B lymphoma patients.

Sept. 1989-June 1993 Rogoff Medical Research Institute, Beilinson Medical Center., Israel
The Laboratory was moved to Felsenstein Medical Research. Center,
Beilinson Campus, Petah Tikva, Israel

June 1993-present Head of Cell Immunology Unit

List of Publications

- L. Rozenszain, D. Fischer, J. Sahar, J. Epstein and B. Kogut*
 Cytochemical Findings in Lymphocytes in blood culture.
 Harefuah, 1967, V.LXXIII n. 6. 193-195.
- A. Eshkol, B. Hardy and C. Pariente-Coriat.
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- 3. **B. Hardy**, D. Danon, A. Eshkol and B. Lunenfeld. Ultrastructural changes in the ovaries of infant mice deprived of endogeneous gonadotrophins and after substitution with FSH. J. Reprod. Fert., 1974, 36: 345-352.
- 4. **B. Hardy**, A. Globerson and D. Danon.
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- B. Hardy, E. Mozes and D. Danon.
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- B. Hardy, E. Skutelsky, A. Globerson and D. Danon.
 Ultrstructural differences between macrophages of newborn and adult mice.
 J. Reticulendothel. Soc., 1976, 19: 291-299.
- 7. E. Skutelsky and **B. Hardy**.
 Regeneration of plasmalemma and surface properties of macrophages.
 Exp. Cell. Res., 1976, 101: 337-345.
- B. Hardy and E. Mozes.
 Expression of T-cell suppressor activity in the immune response to a T-independent synthetic polypeptide in newborn mice.
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- 9. **B. Hardy** and S.L. Schrier. The role of spectrin in erythrocyte ghost endocytosis. Biochem.Biophys.Res.Comm., 1978, 84:1153-1161.
- S.L. Schrier, B. Hardy, K. Bensch, I. Junga and J. Krueger. Red blood cell membrane storage lesion. Transfusion, 1979, 19: 158-165.

11. S.L. Schrier, B. Hardy and K.G. Bensch.

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In: Normal and abnormal red cell membranes. Eds. Lux, SE, Marchesi, VT, Fox, CF. New York. Alan R. Liss, 1979, p. 437-449.

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J. Cell. Biol., 1979, 82: 654-663.

13. S.L. Schrier, B. Hardy, I. Junga and L. Ma.

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14. B. Hardy, J.M. Loew, I. Melchers, D. Charron and S.L. Schrier.

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15. **B. Hardy**, J. Hoffman, and A. Neri.

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Biomedicine, 1982, 36: 372-375.

16. **B. Hardy**, J. Hoffman and Z. Ossimi.

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normal and Gaucher disease.

Biochem. Biophys. Res. Comm., 1984, 120: (2) 325-332.

17. C. Clayberger, T. Uyehara, B. Hardy, K. Eaton, M. Karasek and A.M. Krensky.

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T lymphocyte response to endothelial cells.

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J. Immunol., 1986, 136: 1537-1541.

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20. B. Hardy, Y. Hoffman and S. Chazan.

Use of monoclonal antibodies in the study of Gaucher disease.

Harefuah, 1987, V. CXIII n.9 204-206

- B. Hardy, D. Dotan and A. Novogrodsky
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- D. Huminer, B. Hardy, S. Pitlik.
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- 24. **B. Hardy**, M. Galli, E. Rivlin, L. Goren, and A. Novogrodsky Activation of human an lymphocytes by a monoclonal antibody to B lymphlastoid cells; molecular weight and distribution of binding protein. Cancer Immunology Immunotherapy. 1995,40(6) 376-382
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- M. Shohat, B. Hardy, S. Mannheimer, B. Fisch and B. Shohat A new method for isolation of human antisperm antibodies. ANDROLOGIA 1996, 28 275-279
- 27. **B. Hardy**, R. Kovjazin, A. Raiter, N. Ganor and A. Novogrodsky Immune Stimulatory and anti-tumor properties of anti-CD3 and BAT monoclonal antibodies: A comparative study. Human Antibodies and Hybridomas . In Press.
- 28. **B. Hardy**, R. Kovjazin, A. Raiter, N. Ganor and A. Novogrodsky A lymphocyte-Activating monoclonal antibody induces regression of human tumors in SCID mice. Submitted

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- D. Friedman, B. Hardy, D. Danon and A. Globerson. Immunological status of the newborn mouse spleen. Israel. J. Med. Sci. 1974, 10, 1179.
- 2. **B. Hardy**, A. Globerson and D. Danon.

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- 3. B. Hardy and D. Danon.

A microscopic study comparing the phagocytic function of newborn and adult mouse macrophages.

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Expression of T-cell suppressor activity in the immune response to a T-independent synthetic polypeptide in newborn mice. Scand. J. Immunol. 1977, 6, 705.

6. E. Mozes and B. Hardy.

Development of immune competence of T cells in newborn mice using synthetic polypeptides.

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7. **B.** Hardy and S.L. Schrier.

The role of Spectrin in ghost endocytos.

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12. S.L. Schrier and B. Hardy.

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13. J. Hoffman, A. Neri and B. Hardy.

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14. B. Hardy, J. Hoffman and I. Ossimi.

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15. B. Hardy, Z. Ossimi, K.H. Stenzel and A. Novogrodsky.

Monoclonal antibodies inhibiting accessory function of human B lymphoblastoid cell lines.

Israel Immunological Society 16th Cong. May 1986, p. 19.

16. B. Hardy, M. Galli, E. Rivlin, E. Pras and A. Novogrodsky.

Activation of T lymphocytes by monoclonal antibodies to B lymphoblastoid cells.

Israel J. Med. Science, 1991.

17. M. Shohat, B. Shohat, B. Hardy, S. Manheimer, A. Stein and B. Fisch.

A new method for isolation of pure human antisperm antibodies.

Ann. Meet. of the Israeli Soc. of Reproduction. April 1993, Israel p. 150.

18. B. Hardy, R. Kovjazin, A. Raiter, N. Ganor and A. Novogrodsky

A novel monoclonal antibody with immune -stimulatory and anti-tumor properties.

Human Antibodies and Hybridomas 1996 7 (2) 54 (abstract)